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(54) Title: *OB* RECEPTOR ISOFORMS AND NUCLEIC ACIDS ENCODING THEM

(57) Abstract

The *ob* receptor has numerous isoforms resulting from alternative splicing; three novel isoforms, designated c', f, and g are disclosed. The nucleic acids encoding these isoforms are taught. Also part of the invention are vectors containing the nucleic acid encoding the receptors, host cells transformed with these genes, and assays which use the genes or protein isoforms.

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TITLE OF THE INVENTION

OB RECEPTOR ISOFORMS AND NUCLEIC ACIDS ENCODING THEM

FIELD OF THE INVENTION

This invention relates to *ob* receptor protein isoforms, to DNA and RNA sequences encoding them, and to assays using the receptor isoform proteins.

BACKGROUND OF THE INVENTION

Recently the identification of mutations in several genes involved in the onset of obesity in rodents have been identified. Of particular interest are mutations discovered in the peptide hormone, leptin, which is a component of a novel signal transduction pathway that regulates body weight (Zhang *et al.* 1994, *Nature* 372:425-432; Chen *et al.* 1996, *Cell* 84:491-495). Leptin was initially discovered by the positional cloning of the obesity gene, *ob*, in mice. Two different ob alleles have been identified: one mutation causes the premature termination of the leptin peptide resulting in a truncated protein, and the other mutation changes the transcriptional activity of the *obesity* (*ob)* gene, resulting in a reduced amount of circulating leptin.

There is a correlation between a decrease in the levels of biologically active leptin and the overt obese phenotype observed in *ob/ob* mice. Recombinant leptin has been shown to induce weight loss in the *ob/ob* mouse but not in the diabetic phenotype *db/db* mouse (Campfield *et al.* 1995, *Science* 269: 546-549; Halaas *et al.* 1995, *Science* 269: 543-546; Pellymounter *et al.* 1995, *Science* 269:540-543; Rentsch *et al.* 1995, *Biochem. Biophys. Res. Comm.* 214:131-136; and Weigle *et al.* 1995, *J. Clin. Invest.* 96:2065-2070).

Although the synthesis of leptin occurs in the adipocyte, its ability to decrease food intake and increase metabolic rate appears to be mediated centrally by the hypothalamus. Injection of recombinant leptin into the third ventricle of the brain elicits a similar response as peripheral administration of leptin.

Furthermore, the recent cloning of the human receptor for the leptin, the ob-receptor (OB-R), reveals that it is transcribed in the hypothalamus (Tartaglia *et al.* 1995, *Cell* 83:1263-1271; Stephens *et al.* 1995, *Nature* 377: 530-532). In addition, a mutation that results in premature termination of the long-form of the mouse OB-R, which is preferentially expressed in the hypothalamus, appears to be responsible for the obese phenotype of the *db/db* mouse (Lee *et al.* 1996, *Nature* 379:632-635; Chua *et al.* 1996, *Science* 271:994-996; and Chen *et al.* 1996, *Cell* 84:491-495).

The OB-R from wild type (lean) rats and from rats having the *fatty* mutation (both heterozygous and homozygous *fa*) have been isolated and sequenced. (Patent Application Serial Nos. _____, Attorney Docket Nos. 19642PV and 19642PV2, filed February 22, 1996 and March 22, 1996, which are hereby incorporated by reference.)

Various isoforms of the OB-Rs have also been identified. These isoforms are due to alternative splicing. For example, in the mouse the a form has 5 amino acids following the Lysine at 889; the b form has 273 amino acids after Lysine 889; the c form has 3 amino acids after Lysine 889; and the d form contains 11 amino acids after Lysine 889.

It would be desirable to be able to further experiment with various isoforms in order to better understand obesity, and to be able to clone and produce novel *ob* receptor isoforms to use in assays for the identification of ligands which may be useful in understanding obesity and for its prevention and treatment.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to novel *ob* receptor isoforms designated c', f and g which are substantially free from associated membrane proteins. It also relates to substantially purified *ob* receptor isoform c', f and g proteins. These isoforms are present in various species, including rat, mouse and human.

Another aspect of this invention is to nucleic acids which encode OB receptor isoforms c', f or g. The nucleic acid may be any nucleic acid which can encode a protein, such as genomic DNA, cDNA, or any of the various forms of RNA. Preferably, the nucleic acid is cDNA.

This invention also includes vectors containing a OB-R isoform c', f or g gene, host cells containing the vectors, and methods of making susbstantially pure OB-R isoform c', f or g protein comprising the steps of introducing a vector comprising a OB-R isoform c', f or g gene into a host cell, and cultivating the host cell under appropriate conditions such that OB-R isoform c', f or g is produced. The OB-R isoform c', f or g so produced may be harvested from the host cells in conventional ways.

Yet another aspect of this invention are assays which employ OB-R isoform c', f or g. In these assays, various molecules, suspected of being OB-R isoform c', f or g ligands are contacted with a OB-R isoform c', f or g, and their binding is detected. In this way agonists, antagonists, and ligand mimetics may be identified. A further aspect of this invention are the ligands so indentified.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is the amino acid sequence of wild type rat OB-R.

FIGURE 2 is the cDNA sequence of wild type rat OB-R.

FIGURE 3 is the cDNA sequence encoding rat isoform.

FIGURE 4 is the cDNA specific for Rat isoform c'.

As used througout the specification and claims, the following definitions apply:

"Substantially free from associated membrane proteins" means that the receptor protein is not in physical contact with any membrane proteins.

"Substantially purified OB-receptor isoform c', f or g" means that the protein isoform is at least 90% and preferably at least 95% pure.

"Wild type" means that the gene or protein is substantially the same as that found in an animal which is not considered to have a mutation for that gene or protein.

"*fa*" means that the gene or protein is substantially the same as that found in a rat homologous for the *fatty* mutation.

"Substantially the same" when referring to a nucleic acid or amino acid sequence means either it is the same as the reference sequence, or if not exactly the same, contains changes which do not affect its biological activity or function.

It has been suprisingly found, in accordance with this invention that the OB-R exists in a large variety of isoforms, including three novel ones, form c', f and g. These isoforms apply to all species, but for convenience, throughout the specification and claims, numberings of amino acids and nucleotides will use the rat wild type sequences (FIGURES 1 and 2) as a reference. However, it is to be understood that this invention is not limited to rat wild type proteins and nucleic acids and specifically includes rat (wild type and *fatty*), mouse, and human OB-R isoform c', f and g proteins and nucleic acids.

OB-R isoform f differs from wild type protein in that after the Lysine at position 889 (referring to the rat sequence in FIGURE 1), there are six amino acids, ending at an Asparagine residue at position 895. In the cDNA, the codons are then followed by a Stop codon. One cDNA for rat isoform f is shown in FIGURE 3; this invention specifically includes all various cDNAs encoding an isoform f protein. The superscripted numbers refer to protein position numbers.

Lys^{889} Iso^{890} Met^{891} Pro^{892} Gly^{893} Arg^{894} Asn^{895}

In the human isoform f, Lysine 891 corresponds to the rat Lysine 889, the same six amino acids follow Lysine 889.

In a particularly preferred embodiment of this invention, the OB-R isoform f is from rat origin.

OB-R isoform g differs from the wild type in that it is much shorter that the wild type sequence. The following eighteen amino acids are found at the beginning of the protein with the superscript numbers indicating their position. The Arginine at position 18 is spliced to a large fragment of the wild type molecule, beginning at the Proline at position 166 (in both mouse and human). This isoform then extends for the remainder of the wild type molecule.

Met^{1} Phe^{2} Gln^{3} Thr^{4} Pro^{5} Arg^{6} Ile^{7} Val^{8} Pro^{9} Gly^{10} His^{11} Lys^{12} Asp^{13} Leu^{14} Ile^{15} Ser^{16} Lys^{17} Arg^{18} Pro^{166}. . .

After Pro ^{166}, the remainder of the protein may be the same as wild type, or, alternatively it could also contain another isoform variation, such as isoform a, b, c, d, e, or f.

A particularly preferred embodiment is the rat isoform g.

OB-R isoform c' is similar to the OB-R isoform c which was previously described [Lee *et al.*, *Nature* 379: 632-635]. After Lysine at position 889, it only has three amino acids, Val^{890} Thr^{891} Phe^{892} Stop. As can be seen, isoform c' differs from isoform c in that the final amino acid is phenylalanine rather than valine found in isoform c. Further, there are untranslated sequences in the DNA encoding isoform c' which do not appear to be present in isoform c. The cDNA encoding the rat isoform c' is given in FIGURE 4. In humans, the Val, Thr, Phe follow Lysine 891.

One aspect of this invention is the molecular cloning of these various isoforms of OB-R. The wild type and *fa* receptor proteins contain an extracellular, a transmembrane domain. In the rat, the extracellular domain extends from amino acids 1-830; the transmembrane domain is from amino acids 839-860; and the cytoplasmic domain is from amino acids 860-1162. Similar domains have bene identified for the mouse and human proteins. This

invention also includes isoform c', f and g proteins which lack one or more of these domains. Such deleted proteins are useful in assays for identifying ligands and their binding activity.

In the rat wild type protein, amino acids 1-28 form a signal sequence; thus the mature proteins extend from amino acids 28-1162. The mature protein isoforms form yet another aspect of this invention. This differs somewhat from the signal sequence of 1-22 reported for mouse and human OB-R; the mature mouse and human isoforms form yet another aspect of this invention.

The OB-R isoform c', f or g gene can be introduced into virtually any host cell using known vectors. Preferred host cells include *E. coli* as well as mammalian and yeast cell lines.

One of ordinary skill in the art is able to choose a known vector which is appropriate for a given host cell; generally plasmids or viral vectors are preferred. The OB-R isoform c', f or g gene may be present in the vector in its native form, or it may be under the control of a heterologous promoter, and if desired, one or more enhancers, or other sequences known to regulate transcription or translation. The host cell containing the OB-R isoform c', f or g gene is cultured, and the OB-R isoform c', f or g gene is expressed. After a suitable period of time the OB-R c', f or g isoform protein may be harvested from the cell using conventional separation techniques.

A further aspect of this invention is the use of an OB-R c', f or g isoform in assays to identify OB-R c', f or g isoform ligands. A ligand binds to the OB-R isoform receptor, and *in vivo* may or may not result in an activation of the receptor. Ligands may be agonists of the receptor (i.e. stimulate its activity), antagonists (inhibit its activity) or they may bind with little or no effect upon the receptor activity.

In an assay for ligands, an OB-R isoform of this invention is exposed to a putative ligand, and the amount of binding is measured. The amount of binding may be measured in many ways; for example, a ligand or the OB-R isoform being investigated

may be labeled with a conventional label (such as a radioactive or fluorescent label) and then put in contact with the OB-R isoform under binding conditions. After a suitable time, the unbound ligand is separated from the OB-R isoform and the amount of ligand which has bound can be measured. This can be performed with any of the OB-R isoforms of this invention; alternatively the amount of binding of the various isoforms can be compared. In a competitive assay, both the putative ligand and a known ligand are present, and the amount of binding of the putative ligand is compared to the amount of binding to a known ligand. Alternatively, the putative ligand's ability to displace previously bound known ligand (or vice-versa) may be measured. In yet other embodiments, the assay may be a heterogeneous one, where the OB-R isoform may be bound to a surface, and contacted with putative ligands. Dectection of binding may be by a variety of methods, including labelling, reaction with antibodies, and chomophores.

In another assay, the OB-R isoforms of this invention may be used in a "trans" activation assay. Such assays are described in U.S. Application Serial No. _____, Attorney Docket No. 19686PV, which was filed on April 22, 1996 and which is hereby incorporated by reference. In this assay, a cell which expresses an OB-R isoform of this invention (either naturally or through recombinant means) is transfected with a reporter gene construct comprising a minimal promoter, a leptin activation element and a reporter gene. Transcription of the reporter gene is dependant upon activation of the leptin activation element. Binding of a ligand to the receptor isoform activates the leptin activation element, which then allows transcription of the reporter gene.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Preparation of mRNA and cDNA from rat tissues

Tissues were collected from lean and *fa/fa* Zucker rats and snap frozen in liquid nitrogen. The tissues collected included: hypothalamus, pituitary, lung, liver, kidney, heart, adrenal glands, smooth muscle, skeletal muscle, and adipose tissue. The tissues were homogenized with a Brinkmann Polytron homogenizer in the presence of guanadinium isothiocyanate. mRNA was prepared from hypothalamus, lung, and kidney according to the instructions provided with the messenger RNA isolation kit (Stratagene, La Jolla, CA). cDNA was prepared from approximately 2 µg of mRNA with the SuperScript™ choice system (Gibco/BRL Gaithersburg, MD). The first strand cDNA synthesis was primed using 1 µg of oligo(dT)12-18 primer and 25 ng of random hexamers per reaction. Second strand cDNA sythesis was performed according to the manufacturer's instructions. The quality of the cDNA was assessed by labeling an aliqout ($1/10^{th}$) of the second strand reaction with approximately 1 µCi of [α-^{32}P]dCTP (3000 Ci/mmol). The labeled products were separated on an agarose gel and detected by autoradiography.

EXAMPLE 2

Preparation of a hypothalamic cDNA library

Approximately 3.6 µg of phosphorylated*Bst*XI adapters (Invitrogen, San Diego, CA) were ligated to approximately 3 µg of cDNA prepared as described in Example 1. The ligation mix was then diluted and size-fractionated on a cDNA sizing column (Gibco/BRL Gaithersburg, MD). Drops from the column were collected and the eluted volume from the column was determined. An aliqout from each fraction was analyzed on an agarose gel. Fractions containing cDNA of greater than or equal to 1 kb were pooled and precipitated. The size-fractionated cDNA with the *Bst* XI adapters was ligated into the prokaryotic vector pcDNA II

(Invitrogen, San Diego, CA). The vector (4 µg) was prepared for ligation by first cutting with the restriction endonuclease *Bst* XI, gel purifying the linearized vector, and then dephosphorylating the ends with calf intestinal phosphatase (Gibco/BRL, Gaithersburg, MD) according to the manufacturers instructions. The ligation contained approximately 10-20 ng of cDNA and approximately 100 ng of vector and was incubated overnight at 14°C. The ligation was transformed into 1 ml of XL-2 Blue Ultracompetent cells (Stratagene, La Jolla, CA) according to the manufacture's intructions. The transformed cells were spread on 133 mm Colony/Plaque Screen filters (Dupont/NEN, Boston, MA), plated at a density of 30,000 to 60,000 colonies per plate on Luria Broth agar plates containing 100 µg/ml Ampicillin (Sigma, St. Louis, MO).

EXAMPLE 3

Screening a hypothalamic cDNA library

Colonies on filters were replica plated onto a second filter set. The master filter was stored at 4°C for subsequent isolation of regions containing colonies that gave a positive hybridization signal. The replica filters were grown for several hours at 37°C until colonies were visible and then processed for in situ hybridization of colonies according to established procedures (Maniatis, et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, Cold Spring Harbor, NY, which is hereby incorporated by reference). A Stratalinker (Stratagene, La Jolla, CA) was used to crosslink the DNA to the filter. The filters were washed at 55°C for 2 hours in 2x SSC and 0.5% SDS to remove bacterial debris. Eight to ten filters were then placed in a heat sealable bag (Kapak, Minneapolis, MN) containing 15-20 ml of 1x hybridization solution (Gibco/BRL, Gaithersburg, MD) containing 50% formamide and incubated for 1 hour at 42°C. The filters were hybridized overnight with greater than 1,000,000 cpm/ml of the radiolabeled probe described below in 1x

hybridization buffer (Gibco/BRL, Gaithersburg, MD) containing 50% formamide at 42°C. The probe, a 2.2 kb fragment encoding the extracellular portion of the Ob-R was labeled by random priming with [alpha ^{32}P]dCTP (3000 Ci/mmole, Amersham, Arlington Heights, IL) using redi-prime (Amersham, Arlington Heights, IL). The probe was purified from unincorporated nucleotides using a Probequant G-50 spin column (Pharmacia Biotech, Piscataway, NJ). Filters were washed two times with 0.1x SSC 0.1% SDS at 60°C for 30 min and then subjected to autoradiography. Individual regions containing hybridization positive colonies were lined up with the autoradiogram of the hybridized filter. These were excised from the master filter, and placed into 0.5 ml Luria broth plus 20% glycerol. Each positive was replated at a density of approximate 50-200 colonies per 100 by 15 mm plate and screened by hybridization as previously described. Individual positive colonies were picked and plasmid DNA was prepared from an overnight culture using a Wizard kit (Promega, Madison, WI).

EXAMPLE 4

Amplification of Lean Rat OB-receptor cDNA using PCR

To provide for a probe to screen the hypothalamic cDNA library, the rat OB receptor was initially obtained by PCR using degenerate primers based on the mouse and human OB-receptor amino acid sequences. A set of oligonucleotide primers, were designed to regions with low codon degeneracy. The pairing of the forward primers ROBR 2 (5'-CAY TGG GAR TTY CTI TAY GT-3') and ROBR 3 (5'-GAR TGY TGG ATG AAY GG-3') corresponding to mouse amino acid sequences HWEFLYV and ECWMKG, with reverse primers ROBR 6 (5 '-ATC CAC ATI GTR TAI CC-3'), ROBR 7 (5'-CTC CAR TTR CTC CAR TAI CC-3'), ROBR 8 (5'-ACY TTR CTC ATI GGC CA-3') and ROBR 9 (5'-CCA YTT CAT ICC RTC RTC-3') representing mouse amino acids, GYTMWI, VYWSNWS, WPMSKV, and DDGMKW provided good yields of the appropriately sized products. The fragments of interest

were amplified as long polymerase chain reaction (PCR) products by a modifying the method of Barnes (1994, *Proc. Natl. Acad. Sci.* 91:2216-2220, which is hereby incorporated by reference). In order to obtain the required long PCR fragments, Taq Extender (Stratagene, La Jolla CA) and the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN) were used in combination. The standard PCR reaction mix, in a final volume of 20 µl, contained 5 ng of template (lean rat cDNA), 100 ng of primers, 500 µM dNTPs, 1 X Buffer 3 from the Expand kit, 0.1 µl each of Taq Polymerase and Taq Expander. Reactants were assembled in thin walled reaction tubes.

The amplification protocol was: 1 cycle of 92°C for 30 sec., followed by 32 cycles at 92°C for 30 sec., 45°C for 1 min. and 68°C for 3 min. using a Perkin-Elmer (Norwalk, CT) 9600 Thermal Cycler.

This strategy produced a series of PCR products with the largest being approximately 2.2 Kbp amplified from primers ROBR 2 and ROBR 9. These products were subcloned for DNA sequence analysis as described below. The insert was excised from the cloning vector with the restriction endonuclease *Eco* RI, and fragments were separated from the vector by agarose gel electrophoresis. The fragments were eluted from the gel using a Prep-A-Gene kit (BioRad, Richmond CA) according to the manufacturer's instructions and radiolabeled as described above.

EXAMPLE 5

Subcloning of PCR products

PCR products of the appropriate size were prepared for subcloning by separation on an agarose gel, excising the band, and extracting the DNA using Prep-A-Gene (BioRad, Richmond, CA). PCR products were ligated into pCR™II (Invitrogen, San Diego, CA) according to the instructions provided by the manufacturer. The ligation was transformed into INVaF' cells and plated on Luria-Bertani plates containing 100 µg/ml ampicillin and X-Gal (32 µl of

50 mg/ml X-Gal (Promega, Madison, WI). White colonies were picked and grown overnight in Luria-Bertani broth plus 100 µg/ml ampicillin. Plasmid DNAs were prepared using the Wizard miniprep kit (Promega, Madison, WI). Inserts were analyzed by digesting the plasmid DNA with EcoRI and separating the restriction endonulease digestion products on an agarose gel.

Plasmid DNA was prepared for DNA sequencing by ethanol precipitation of Wizard miniprep plasmid DNA and resuspending in water to achieve a final DNA concentration of 100 µg/ml. DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS. The initial DNA sequence analysis was performed with M13 forward and reverse primers, subsequently primers based on the rat OB-R sequence were utilized. Following amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). DNA sequence data was analyzed with the Sequencher program.

WHAT IS CLAIMED IS:

1. *Ob*-receptor (OB-R) isoform c', f or g, sustantially free from associated proteins.

2. An OB-R isoform according to Claim 1 which is substantially pure.

3. An OB-R isoform according to Claim 1 which is a c' isoform.

4. An OB-R isoform according to Claim 1 which is an f isoform.

5. An OB-R isoform according to Claim 1 which is a g isoform.

6. An OB-R isoform according to Claim 1 which is from a rat.

7. An OB-R isoform according to Claim 6 which is from a wild-type rat.

8. An OB-R isoform according to Claim 6 which is from a *fatty* rat.

9. An OB-R isoform according to Claim 3 which is human.

10. An OB-R isoform according to Claim 4 which is human.

11. An OB-R isoform according to Claim 5 which is human.

12. An OB-R isoform according to Claim 3 which is from a mouse.

13. An OB-R isoform according to Claim 4 which is from a mouse.

14. An OB-R isoform according to Claim 5 which is from a mouse.

15. A nucleic acid encoding an OB-R of Claim 1.

16. A nucleic acid according to Claim 15 which is a cDNA.

17. A vector comprising a nucleic acid which encodes an OB-R of Claim 1.

18. A vector according to Claim 17 which is a plasmid.

19. A host cell containing a vector according to Claim 17.

20. A host cell according to Claim 19 which is *E. coli*, a mammalian cell, or a yeast cell.

21. An assay to determine if a putative ligand binds to an OB-R isoform c', f or g comprising: contacting the putative ligand with an OB-R isoform c', f or g, and determining if binding has occurred.

22. An assay according to Claim 17 wherein the ligand is labeled.

23. An assay to determine if a putative ligand binds to an OB-R isoform c', f or g which is a trans-activation assay.

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  1  MTCQKFYVVL LHWEFLYVIT ALNLAYPTSP WRFKLFCAPP STTDDSFLSP
 51  AGVPNNTSSL KGASEALVEA KFNSTGIYVS ELSKTIFHCC FGNEQGQNCS
101  ALTGNTEGKT LASVVKPLVF RQLGVNWDIE CWMKGDLTLF ICHMEPLLKN
151  PFKNYDSKVH LLYDLPEVID DLPLPPLKDS FQTVQCNCSV RECECHVPVP
201  RAKVNYALLM YLEITSAGVS FQSPLMSLQP MLVVKPDPPL GLRMEVTDDG
251  NLKISWDSQT KAPFPLQYQV KYLENSTIVR EAAEIVSDTS LLVDSVLPGS
301  SYEVQVRSKR LDGSGVWSDW SLPQLFTTQD VMYFPPKILT SVGSNASFCC
351  IYKNENQTIS SKQIVWWMNL AEKIPETQYN TVSDHISKVT FSNLKATRPR
401  GKFTYDAVYC CNEQACHHRY AELYVIDVNI NISCETDGYL TKMTCRWSPS
451  TIQSLVGSTV QLRYHRRSLY CPDNPSIRPT SELKNCVLQT DGFYECVFQP
501  IFLLSGYTMW IRINHSLGSL DSPPTCVLPD SVVKPLPPSN VKAEITINTG
551  LLKVSWEKPV FPENNLQFQI RYGLNGKEIQ WKTHEVFDAK SKSASLPVSD
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FIG. 1A

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601  LCAVYVVQVR CRRLDGLGYW SNWSSPAYTL VMDVKVPMRG PEFWRIMDGD
651  ITKKERNVTL LWKPLMKNDS LCSVRRYVVK HRTAHNGTWS QDVGNQTNLT
701  FLWAESAHTV TVLAINSIGA SLVNFNLTFS WPMSKVNAVQ SLSAYPLSSS
751  CVILSWTLSP NDYSLLYLVI EWKNLNDDDG MKWLRIPSNV NKYYIHDNFI
801  PIEKYQFSLY PVFMEGVGKP KIINGFTKDD IAKQQNDAGL YVIVPIIISS
851  CVLLLGTLLI SHQRMKKLFW DDVPNPKNCS WAQGLNFQKP ETFEHLFTKH
901  AESVIFGPLL LEPEPVSEEI SVDTAWKNKD EMVPAAMVSL LLTTPDSTRG
951  SICISDQCNS ANFSGAQSTQ GTCEDECQSQ PSVKYATLVS NVKTVETDEE
1001 QGAIHSSVSQ CIARKHSPLR QSFSSNSWEI EAQAFFLLSD HPPNVISPQL
1051 SFSGLDELLE LEGNFPEENH GEKSVYYLGV SSGNKRENDM LLTDEAGVLC
1101 PFPAHCLFSD IRILQESCSH FVENNLNLGT SGKNFVPYMP QFQSCSTHSH
1151 KIIENKMCDL TV
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FIG. 1B

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   1  TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
  51  ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
 101  TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACTT AACCTGGCCT
 151  ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
 201  ACTGATGACT CCTTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
 251  TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG
 301  GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
 351  AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
 401  GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
 451  ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
 501  CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGGT
 551  TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
 601  CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA
 651  TGCGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
```

FIG. 2A

```
 701  GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
 751  TGTCACTGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG
 801  CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA
 851  AACAAAAGCA CCATTTCCAC TTCAATATCA GGTGAAATAT TTAGAGAATT
 901  CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
 951  GTAGACAGCG TGCTTCCTGG GTCTTCATAC GAGGTCCAGG TGAGGAGCAA
1001  GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT
1051  TTACCACACA AGATGTCATG TATTTTCCAC CCAAAATTCT GACGAGTGTT
1101  GGATCCAATG CTTCCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151  CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201  AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251  AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA
1301  CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
```

FIG. 2B

```
1351  TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA
1401  ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC
1451  TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT
1501  CTATTCGTCC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC
1551  TTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601  GTGGATCAGG ATCAACCATT CTTTAGGTTC ACTTGACTCT CCACCAACGT
1651  GTGTCCTTCC TGACTCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701  GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751  AGTCTTTCCA GAGAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG
1801  GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851  TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT
1901  TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951  CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA
2001  TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
```

FIG. 2C

2051 CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA

2101 GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT

2151 GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC

2201 TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA

2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC

2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC

2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA

2401 ATGATGATGA TGGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG

2451 TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT

2501 TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT

2551 TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC

2601 ATTGTACCGA TAATTATTTC CTCTTGTGTC CTGCTGCTCG GAACACTGTT

2651 AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC

FIG. 2D

```
2701  CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GCCTGAAACA
2751  TTTGAGCATC TTTTTACCAA GCATGCAGAA TCAGTGATAT TTGGTCCTCT
2801  TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851  GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901  ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTAA
2951  CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001  AGTGTCAGAG TCAACCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051  AAAACAGTGG AAACTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101  CCAGTGCATC GCCAGGAAAC ATTCCCCACT GAGACAGTCT TTTTCTAGCA
3151  ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA
```

FIG. 2E

```
3201  CCCAATGTGA TTTCACCACA ACTTTCATTC TCAGGGTTGG ATGAGCTTTT
3251  GGAACTGGAG GGAAATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301  ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
3351  ACTGATGAGG CAGGGGTATT GTGCCCATTC CCAGCTCACT GTCTGTTCAG
3401  TGACATCAGA ATCCTCCAGG AGAGTTGTTC ACACTTTGTA GAAAATAATT
3451  TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT
3501  CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAATA AGATGTGTGA
3551  CTTAACTGTG TAATCTTGTC CAAAAACTTC CAGGTTCCAT TCCAGTAGAG
3601  TGTGTCATGT ATAATATGTT CTTTTATAGT TGTGGGTGGG AGAGAAAGCC
```

FIG. 2F

```
  1  TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
 51  ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAGAAAT TCTATGTGGT
101  TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACTT AACCTGGCCT
151  ATCCAACCTC TCCCTGGAGA TTTAAGCTGT TTTGTGCGCC ACCGAGTACA
201  ACTGATGACT CCTTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
251  TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATTT AATTCAACTG
301  GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
351  AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
401  GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
451  ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
501  CATATGGAAC CATTACTTAA GAACCCCTTC AAGAATTATG ACTCTAAGGT
551  TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
601  CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA
```

FIG. 3A

```
651   TGCGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
701   GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
751   TGTCACTGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG
801   CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA
851   AACAAAAGCA CCATTTCCAC TTCAATATCA GGTGAAATAT TTAGAGAATT
901   CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
951   GTAGACAGCG TGCTTCCTGG GTCTTCATAC GAGGTCCAGG TGAGGAGCAA
1001  GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTTA CCTCAACTCT
1051  TTACCACACA AGATGTCATG TATTTTCCAC CCAAAATTCT GACGAGTGTT
1101  GGATCCAATG CTTCCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151  CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201  AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251  AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA
```

FIG. 3B

```
1301  CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
1351  TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA
1401  ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC
1451  TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT
1501  CTATTCGTCC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC
1551  TTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601  GTGGATCAGG ATCAACCATT CTTTAGGTTC ACTTGACTCT CCACCAACGT
1651  GTGTCCTTCC TGACTCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701  GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751  AGTCTTTCCA GAGAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG
1801  GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851  TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT
```

FIG. 3C

```
1901  TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951  CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCTATGAG AGGGCCTGAA
2001  TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
2051  CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
2101  GGTATGTGGT GAAGCATCGT ACTGCCCACA ATGGGACATG GTCACAAGAT
2151  GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201  TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251  ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301  AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351  ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401  ATGATGATGA TGGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAACAAG
2451  TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
```

FIG. 3D

```
2501  TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT
2551  TCACCAAAGA TGATATCGCC AAACAGCAAA ATGATGCAGG GCTGTATGTC
2601  ATTGTACCGA TAATTATTTC CTCTTGTGTC CTGCTGCTCG GAACACTGTT
2651  AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
2701  CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAAA GATAATGCCTG
2751  GCAGAAATTA GAGGATATAG AGTGGATGCC GTCAAATGCC TTTAGACTCT
2801  GGCTTCCCTG GCTGTCTCAC ATCTCCCCTA TTGGAGCTAA GTGTGGTGCT
2851  GTATTTAGCA GGGTATCTGG CAGATATTTT AAATTAATTG AAATATCACC
2901  CTAAATTTCC AGATTCTGGT AAACTGAAGT GAATTTCAGA AATTATTGTA
2951  TTTATGTGTG TGCACATATG TGTGCAGGTA CCCACCGAAA TCTGCAGAGG
3001  CATCAGATGC CCCAGAGCTG GAACTGACAG TTGTGAGCCT GATATGAGTT
3051  CTGGGAATGA GCTCAGTCCT CTGGAAGAGC TGCAAGCACT ATTAACTGCT
```

FIG. 3E

```
3101  GAGCCATCTT TTCAGTCCCT CATGTATAGA TTAAAAAAAA TTGGGGTTTG
3151  AAGATCCTCA TTTGTGAGAA ATTCCTTCTT ACCTTTGCAA ACACTTTTTC
3201  TCATTTTTAG TATATGTATT CATATTTTAC TGTCTCATTT TCAATATATG
3251  TGGTCACAGT TTTTAAGTAT TTCTAAGGCA TAACAAAGAT GTAATATTAA
3301  GAATAAATAA AAGAATAAAT CAATAATCCA GATGGTAGTG ACAGACACCT
3351  TTAATCCCAG TACTAAGGAG ACAGAGATAG GTAAATCTGT ATGAATTTGA
3401  GACACGCCTG TTCTACAAAG AAATTTCAGG ACATCTAGGG GTATCCACAA
3451  AGAAACACTG TCTCAAAAAA TGCCAAACAA TCAAAAAAAA AAAA
```

FIG. 3F

```
  1  GTCACTTTTT AAGTATTTAC CCAAGATATC TAAGGTTGCA GTTTAGATAC
 51  TCTATTACAT AGAGATCTTT AAACATCTTT AAAAGGCTTT ATTTTGTCCT
101  GTTCACTTTA TTAATCCCGT TTATCCTTTG TCTATAGCAA TAGCTGGGTT
151  TTGGATTTGA TCAGAGGAAA CAAAGTTCAG TCATTTATCA CATGAGAGTT
201  GACAAGGTGT CTTTTTTTTT TCTCGTCACT GTACATAAAA AAATAAATAC
251  TACAAGAGGA AGGAACATTG TAGATGGAGA ATAGATAACT GACTAAAAGG
301  GCTTTCTTTA GTCAAAAAGT TTAGGATCAA TATTATGAGT TTCTGATATT
351  CAATATTTCA CCATGACTTA CAAGTACAGT GTTGTTTTT
```

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07521

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.5, 252.3, 320.1, 7.1, 7.2; 536/23.5; 530/350, 351; 514/2,8,12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	TARTAGLIA et al . Identification and expression cloning of a Leptin Receptor, OB-R. Cell. 29 December 1995, Vol. 83, pages 1263-1271, see entire document.	1-5, 9-20 ----- 1-23
X --- Y	CHUA et al. Phenotypes of Mouse *diabetes* and Rat *fatty* due to mutations in the OB (Leptin) Receptor. Science. 16 February 1996, Vol. 271, see pages 994-996.	1-8, 12-20 ----- 1-23
X --- Y	CHEN et al. Evidence that the diabetes gene encodes the Leptin Receptor: Identification of a mutation in the Leptin Receptor gene in *db/db* mice. Cell. 09 February 1996, Vol. 84, pages 491-495, see entire document.	1-5, 12-20 ----- 1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search
19 JUNE 1997

Date of mailing of the international search report
11 JUL 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07521

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	CIOFFI et al. Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. Nature Medicine. May 1996, Vol. 2, No. 5, pages 585-589, see entire document.	1-5, 9-20 ----- 1-23
X	WO 96/08510 A1 (PROGENITOR, INC.) 21 March 1996 (21.03.96), see the figures and claims.	1-23
X --- Y	LEE et al. Abnormal splicing of the leptin receptor in *diabetic* mice. Nature. 15 February 1996, Vol. 379, pages 632-635, see entire document.	1-5, 12-20 ----- 1-23
X	HODGSON J. Receptor screening and the search for new pharmaceuticals. Bio/Technology. September 1992, Vol. 10, pages 973-997, see entire document.	21-23
X	CA 2,104,996 A1 (BEHRINGWERKE AKTIENGESELLSCHAFT) 01 March 1994 (01.03.94), see the claims.	21-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07521

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12P 21/00; C12N 1/20, 15/00; G01N 33/53; C07H 21/04; C07K 1/00, 14/52; A61K 45/05, 38/19, 38/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 69.5, 252.3, 320.1, 7.1, 7.2; 536/23.5; 530/350, 351; 514/2,8,12